cell constituents, such as DNA, cytochemical techniques such as the Feulgen stain were sufficient to demonstrate a unique locus. de Duve agreed that cytochemical staining could provide a valuable test of the results of fractionation, but was unwilling to restrict fractionation studies to results that could be vindicated by histochemistry.

By the 1950s cell fractionation was generally regarded as providing reliable evidence of the enzyme composition of cell organelles and thus of their function. Yet skeptics remained. James Danielli, for example, doubted that the results of fractionation were reflective of the activities in living cells: "so far there has been an almost complete lack of proof that the bodies isolated are in the same condition as in the intact cells" (1953, p. 7–8). F. K. Sanders offered a similarly negative assessment:

> except in certain well-defined cases, little evidence has been offered that such fractions are in fact identical with known cellular structures. Moreover, it cannot be assumed that because a certain enzyme is found in isolated 'nuclei' or 'mitochondria' it is, in fact, present in this location in the living cell. Cells are highly complex colloidal systems, in which the distribution of substances between the different parts of the system is likely to be altered by procedures far milder than those [in cell fractionation]. (1951, p. 24)[14]

In the next chapter we will see that even while grounds for skepticism remained, and before a theoretical understanding of the process of fractionation had developed, researchers relied on cell fractionation in developing models of cell mechanisms. That this technique was producing (a) determinate results that (b) to some extent corresponded to results from other techniques and (c) fit into a developing mechanistic model of the cell was

[14] Even as enthusiastic an advocate of cell fractionation as de Duve expressed caution: "As a bridge between the fields of cytology and biochemistry, it [differential centrifugation] offers tremendous possibilities which even the most carefully worked out techniques of cytochemistry could never have been expected to fulfill. It must be remembered, however, that the application of differential centrifugation is fraught with many technical difficulties and open to a large number of errors. The methods that have been worked out today represent significant improvements over the earlier ones, but much remains to be done to augment their accuracy and selectivity. For this purpose it is important to have in mind the theoretical basis of the technique as well as the various factors of practical nature which have been found to affect the results. . . . The limitations of differential centrifugation become particularly severe when the technique is applied to the study of tissue enzymes. It is now quite clear that the observed partitions provide only the roughest sort of information concerning the true intracellular distributions of enzymes. They can only be considered as clues which have to be followed by many additional experiments in order to arrive at their real significance" (de Duve & Berthet, 1954).